A REVIEW OF METHODS FOR DETECTION OF THE PSYCHROTROPHIC FOODBORNE PATHOGENS *LISTERIA MONOCYTOGENES* AND *AEROMONAS HYDROPHILA*[1]

SAMUEL A. PALUMBO

ABSTRACT

The detection of the psychrotrophic foodborne pathogens Listeria monocytogenes *and* Aeromonas hydrophila *in food depends on the use of various selective media designed specifically for their isolation. These selective media, which contain combinations of dyes, antibiotics, and other inhibitory substances, restrict the background microflora while permitting the desired organism (either* L. monocytogenes *or* A. hydrophila*) to form characteristic colonies. Since the selective media are not completely specific, confirmation tests specific to* L. monocytogenes *or* A. hydrophila *are used to verify the identity of the respective isolates. It has been observed that the inhibitory substances used will not permit injured (stressed) cells to form colonies and special techniques are needed to recover injured cells. The present techniques, while not ideal, do allow for a reasonably quantitative estimate of any* L. monocytogenes *or* A. hydrophila *present in a food.*

INTRODUCTION

Traditionally, refrigeration (holding food at 5°C) has been considered adequate to keep foods safe from foodborne pathogens. It has been known for almost 100 years that various psychrotrophic/psychrophilic microorganisms can grow in refrigerated foods and spoil them. However, in recent years, observations were made which indicate that there are various foodborne pathogens which are capable of growth at 5°C (Palumbo 1986). In fact, methods for the isolation of organisms such as *Listeria monocytogenes* and *Yersinia enterocolitica* often involve a cold enrichment step, that is, holding the suspect material at 5°C for long periods to

increase the numbers of these two bacteria and suppress other organisms present. The occurrence of foodborne pathogens capable of growth at 5°C has assumed greater significance since refrigeration has become a major means of preserving foods and maintaining product quality and shelf life. Further, the ability to easily detect and quantitatively recover these psychrotrophic foodborne pathogens also assumes greater importance.

The recovery of pathogenic bacteria from foods is different from and more difficult than the recovery of the same organisms from clinical specimens. This difference is based on the following observations and generalities: (1) In clinical specimens, the organism in question is usually present in high numbers and often is the only organism present in the specimen. Foods often contain a microflora which is physiologically similar to the organism in question. (2) In clinical specimens, the organisms are actively growing under optimal conditions; this is in contrast to foods, especially processed foods, where the organisms present may have been damaged (injured/stressed) by the food processing operations. (3) The clinical microbiologist is generally interested simply in determining the presence or absence of the organism and thus can score the specimen as plus or minus. On the other hand, food microbiologists are concerned not only about the presence of the pathogen, but also the total number of the particular organism present since the risk to the consumer is directly related to the number present in the food.

The food microbiologist often will use clinical procedures, especially media devised for clinical use, as a starting point for recovery of pathogens from food. These clinical media are then refined and specifically tailored to the requirements of the food microbiologist. Media for the recovery of *L. monocytogenes* and *Aeromonas hydrophila* from foods reflect their clinical origins.

The purpose of this paper is to review research on the development of methods for the isolation of *L. monocytogenes* and *A. hydrophila* from foods, procedures to verify the identity of isolates and to review how the presence of injured (stressed) cells influences their quantitative recovery.

ISOLATION AND IDENTIFICATION OF *L. MONOCYTOGENES*

Numerous procedures and media have been proposed for the isolation of *L. monocytogenes* from foods (reviewed by Cassiday and Bracket 1989). An outline of these procedures and selective agents is given in Table 1. It should be noted that most media contain more than one selective agent. The selective agents listed in Table 1 have various functions. (A) One is as an inhibitor and indicator, e.g., potassium tellurite. *L. monocytogenes* reduces tellurite to metallic tellurium and thus forms black colonies on tellurite-containing media; in addition, potassium tellurite inhibits Gram-negative bacteria. (B) A second is as an indicator

TABLE 1.
SELECTIVE AND DIFFERENTIAL AGENTS AND PROCEDURES TO ISOLATE AND ENUMERATE *LISTERIA MONOCYTOGENES* IN FOODS

Selective agent	Medium	Reference to use of agent or technique to isolate *L. monocytogenes* from food or environmental samples
-phenylethanol, lithium chloride, glycine (glycine anhydride)	McBride Listeria agar	McBride and Girard 1960
	Lithium chloride-phenylethanol-moxalactam agar (LPM)	Lee and McClain 1986
-nalidixic acid	Modified Vogel Johnson agar (MVJ)	Buchanan *et al.* 1987
-moxalactam	MVJ	Buchanan *et al.* 1987
	LPM	Lee and McClain 1986
-ceftazidime	Acriflavin-ceftazidime agar (AC)	Bannerman and Bille 1988
	Al-Zoreky-Sandine listeria medium (ASLM)	Al-Zoreky and Sandine 1990
-cycloheximide	Enrichment broth (EB)	Lovett *et al.* 1987
-Bacitracin	MVJ	Buchanan *et al.* 1987
Polymyxin B	Modified Despierres agar (MDA)	Golden *et al.* 1988
cyclohexanedione	cyclohexanedione-nalidixic acid-phenylethanol agar	Loessner *et al.* 1988
Latamoxef	Columbia agar base (CA)	Curtis *et al.* 1989
cefotetan	CA	Curtis *et al.* 1989
fosfomycin	CA	Curtis *et al.* 1989

TABLE 1
(CONTINUED)

Selective agent	Medium	Reference to use of agent or technique to isolate *L. monocytogenes* from food or environmental samples
-Acriflavin	AC agar	Bannerman and Bille 1988
	Listeria enrichment broth (LEB)	Donnelly and Baigent 1988
-Potassium thiocyanate	thiocyanate nalidixic broth	Watkins and Sleath 1981
-guanofuracin	—	McBride and Girard 1960
Dyes	MDA	Golden *et al.* 1988
Differential agent		
-esculin and ferric ammonium citrate	Fraser broth Columbia agar base ASLM	Fraser and Sperber 1988 Van Netten *et al.* 1988 Al-Zoreky and Sandine 1990
Both -Potassium tellurite	MVJ	Buchanan *et al.* 1987
Technique or procedure		
-cold enrichment	tryptose broth	Gray *et al.* 1948
-enrichment broth primary	EB	Lovett *et al.* 1988
	LEB	Donnelly and Baigent 1988
secondary	Modified LEB	McClain and Lee 1988
Henry illumination	LPM lithium chloride-ceftazidime agar	McClain and Lee 1988 Lachica 1990b

system for *L. monocytogenes*. *L. monocytogenes* can hydrolyze esculin and the hydrolysis product reacts with ferric ammonium citrate to yield a dark color. The esculin reaction by *L. monocytogenes* has formed the basis of both an enrichment broth (Fraser and Sperber 1988) and a plating medium (Van Netten *et al.* 1989). (C) The rest of the selective agents (top portion of Table 1) function to inhibit the background microflora of foods. These selective agents and the respective media they are used in vary in how well they recover *L. monocytogenes* from food. Quantitative recovery of the organism was a function of the food itself and the background microflora (Brackett and Beuchat 1989; Cassiday *et al.* 1989a and b; Dominguez *et al.* 1988; Lowry and Tiong 1989). For example, based on relative recovery and ease of recognizing and counting colonies of *L. monocytogenes*, Cassiday *et al.* (1989b) found that LPM agar was most suitable for use on dry- and country-cured hams, while DRIA was most suitable for raw oysters. Further, Lowry and Tiong (1989) compared the USDA/FSIS method (developed originally for meat and meat products), the FDA method (developed for dairy products), and the CDC cold enrichment (developed for cheese) for the isolation of *L. monocytogenes* from meat tissues. They found that the USDA/FSIS method was the most sensitive for meat tissues and could recover the organism when present at as few as one cell per gram of food.

As indicated above, the selective agents are generally added to plating media, dilutions of a food slurry surface plated, and "typical" *L. monocytogenes* counted or scored. However, in many instances, low numbers of *L. monocytogenes* and high numbers of background microflora are present in the food. In these cases, various enrichment systems have been devised (bottom, Table 1). The best known system for *L. monocytogenes* is cold enrichment. In this procedure, samples of contaminated material are placed in a non-restrictive broth (tryptose broth) and held at 5° for periods of several days to several weeks. After this refrigerated holding, portions are plated and *L. monocytogenes* can be isolated in contrast to negative isolation of the non-cold enriched material. As discussed by Cassiday and Bracket (1989), cold enrichment is extremely useful for the isolation of *L. monocytogenes* from various foods. However, the lengthy periods of holding in the cold precludes its use in quality control by the food industry.

In addition to cold enrichment, which utilizes a non-restrictive broth, enrichment broths containing various selective agents have been devised (Cassiday and Brackett 1989). These selective enrichments are formulated to contain nutrients to insure good growth of *L. monocytogenes* and selective agents to restrict the growth of the background microflora of the food. Examples of these include: UVM broth (Donnelly and Baigent 1986) which contained 40 mg/L nalidixic acid and 20 mg/L acriflavin·HCl in a base containing protease peptone, tryptone, and Lab-Lemco powder and was designed to suppress *Staphylococcus aureus* and contaminants of raw milk; EB (Lovett *et al.* 1987) designed for dairy products

and contained nalidixic acid (40 mg/L), acriflavin·HCl (15 mg/L) and cycloheximide (50 mg/L) in a Trypticase soy broth-yeast extract base; and the primary and secondary broths of McClain and Lee (1988). The primary broth was UVM with the nalidixic acid level reduced to 20 mg/L; the secondary broth was the primary broth with the acriflavin content increased to 25 mg/L. They found the combination of these primary and secondary broths useful for meats and meat products. After an appropriate incubation period, portions of the enrichment broth are plated onto selective media and typical colonies of *L. monocytogenes* are scored.

Some further modifications have been introduced intc the enrichment procedures. Doyle and Schoeni (1986, 1987) incubated their enrichment broths in a microaerophilic atmosphere (5% O_2, 10% CO_2, and 85% N_2 before streaking onto a selective medium. They found that this procedure showed promise for some samples of soft, surface-ripened cheese as well as liver and brain tissue. Lovett (1988) added a brief treatment of the enrichment broth (after incubation) with dilute KOH followed by streaking on an appropriate selective medium. This FDA procedure appears quite useful in isolating *L. monocytogenes* from dairy products. One further modification was introduced by McClain and Lee (1988). In their work, they added a second selective enrichment broth and found a much improved isolation of *L. monocytogenes* from meat.

One method which has proved useful in isolating and identifying colonies of *L. monocytogenes* is the Henry illumination technique [see McClain and Lee (1988) for details of the physical arrangement of colony, light, mirror, and microscope]. Lachica (1990b) recently introduced a simplification of the Henry technique which shows promise and provides for a more precise and convenient use of the procedure. As summarized by Cassiday and Brackett (1989), colonies of *L. monocytogenes* appear pearlescent blue on clear media such as LPM or McBride Listeria agars and often a few colonies of the organism can be identified on plates heavily contaminated with background microflora. However, the technique is difficult to use and depends on subjective evaluation which is not necessary when differential selective media are used.

As suggested by Cassiday and Brackett (1989) in their review of selective agents and media, none seems to be completely effective. Because other bacteria can form colonies on these media, "typical" *L. monocytogenes* colonies must be verified as *L. monocytogenes*. This is particularly necessary with foods containing a natural microflora of organisms biochemically similar to *L. monocytogenes* such as staphylococci and streptococci. Lovett (1988) has suggested an identification scheme based on several traits of *L. monocytogenes* (see Table 2). It is important to observe the hemolysin reaction of the culture, especially if it is CAMP test-positive (hemolysis enhanced by cross-streak of *Staphylococcus aureus*). Blanco *et al.* (1989) have suggested a readily incorporated step in the

TABLE 2
BIOCHEMICAL AND PHENOTYPIC TRAITS OF *LISTERIA MONOCYTOGENES*[z]

Gram +, catalase + rod
motility + (culture grown at 30°C or below)
MR-VP +
urease +
nitrate reductase -
H_2S - (in TSI, *L. monocytogenes* gives an acid/acid pattern)
carbohydrate fermentation (in purple broth base)
xylose -
rhamnose +
mannitol -
hemolysin Beta
CAMP test + enhanced beta hemolysis when cross-streaked with culture of *S. aureus*
mouse pathogenicity +

[z] Lovett (1988).

isolation procedure for determining the hemolysin reaction. In their procedure, food samples are surface plated on typical Listeria selective media (e.g., modified McBride agar, lithium chloride-phenylethanol-moxalactam agar, Listeria Selective agar, etc.) and plates incubated 48 h at 37°C. After this period, "typical" *L. monocytogenes* are identified and scored; then an overlay of sheep red blood cells is carefully poured over the colonies and the plates are incubated for an additional 14 h at 37°C. Hemolysis is readily determined after this additional period of incubation; they feel that when hemolysis is combined with catalase, tellurite reduction, and esculin hydrolysis from colonies on certain selective media, an almost confirmed identification of isolates as *L. monocytogenes* is assured. As suggested by Lovett (1988), mouse pathogenicity testing should be done only after the results of the CAMP test are known. Lovett (1988) also recommends the use of fresh, known *L. monocytogenes* as positive or negative controls for each test.

Lachica (1990c) has described a scheme for same-day identification of colonies of *L. monocytogenes*, especially applicable when combined with use of a newly

developed plating medium (lithium chloride-ceftazidime agar) (Lachica 1990a). Specifically, his rapid scheme depends on use of a large inoculum (from a suspect colony) for a rapid hemolysin/CAMP plate test and rapid sugar fermentation reaction test as well as rapid test for motility, Gram reaction, and catalase. His scheme appeared to function well on a series of naturally contaminated foods including oysters, Brie cheese, chicken roll, pork sausage and precooked sliced beef.

Various rapid biochemical test procedures are available to identify *L. monocytogenes* and others species in the genus *Listeria*. Kerr *et al.* (1990) evaluated the Mast ID and API 50CH systems and found both were equally successful; however, the Mast ID is less expensive and allows for an easier screening of larger numbers of cultures.

Two recent papers have described some newly developed methods to type and identify *L. monocytogenes* isolates (Bibb *et al.* 1990; Vogt *et al.* 1990). Vogt *et al.* (1990) used isoenzyme and ribosomal RNA typing to establish identity between a raw milk isolate and the human case isolate. In the second method, Bibb *et al.* (1990) determined that multilocus enzyme electrophoresis was useful for studying the epidemiology of listeriosis.

As with the isolation of other pathogens from foods as well as determination of the total bacteriological quality of a food, alternative/rapid methods for the determination of *L. monocytogenes* in foods have been proposed. Some of these are listed in Table 3. The use of the first three methods was compared to the standard FDA procedure by Heisick *et al.* (1989) who observed that all techniques appeared to work equally well on milk samples, but poorly on fresh vegetables, probably because of the low numbers of *Listeria* spp. in the presence of the mixed microflora of the produce. Beumer and Brinkman (1989) and Comi *et al.* (1990) compared the ELISA technique with standard culture techniques for use on various cheese and meat products; they observed faster detection of *L. monocytogenes* and good correlation with cultural methods. The recently described polymerase chain reaction for *L. monocytogenes* is specific for a portion of listerolysin O, an important virulence factor in the organism. The method (Bessesen *et al.* 1990) appears to function well in detecting *L. monocytogenes* in the presence of other species of *Listeria* as well as other non-Listeria species. Use of change in capacitance was described by Phillips and Griffiths (1989). In this technique, material is placed in a *Listeria*-selective broth (containing nalidixic acid) and the capacitance signal read at intervals during incubation. The method can give a signal characteristic of *Listeria* spp. McLauchlin and Pini (1989) have described the use of monoclonal antibodies in a direct immunofluorescence test to detect *L. monocytogenes* in soft cheese. Their procedure compared well with conventional techniques.

TABLE 3.
NEWER METHODS TO DETECT *L. MONOCYTOGENES* IN FOODS

–ELISA procedure (Organon Teknika)[x]	monoclonal antibody
–Gene TRAK DNA probe[x]	16S rRNA sequence
–FDA probe technique[x]	hemolysin gene
–Polymerase chain reaction[y]	
–electrical method[z]	change in capacitance signal
–Direct immunofluorescence test*	

[x]Heisick *et al.* (1989).
[y]Bessesen *et al.* (1990).
[z]Phillips and Griffiths (1989).
*McLauchlin and Pini (1989).

ISOLATION AND IDENTIFICATION OF *A. HYDROPHILA*

As in the case of isolation of *L. monocytogenes*, much of the early work on isolation of *A. hydrophila* was done by clinical microbiologists. von Graevenitz and Bucher (1983) have reviewed some of the media useful for isolating *A. hydrophila* from fecal specimens. A partial list of the inhibitory and differential agents in these media is given in Table 4. They indicated that trypticase soy medium with ampicillin, inositol-brilliant green-bile salts agar, dextrin fuchsin-sulfite agar, xylose-sodium desoxycholate-citrate agar, and Pril-xylose ampicillin agar were suitable for isolating *A. hydrophila* from stool specimens.

TABLE 4.
DIFFERENTIAL AND SELECTIVE AGENTS USED IN MEDIA TO ISOLATE *A. HYDROPHILA*[z]

Selective agents	Differential agents
sodium sulfite	dextrin
fuchsin	toluidine blue (DNase)
ampicillin	glycogen
Pril	xylose
citrate	
novobiocin	
bile salts/sodium desoxycholate	

[z]von Graevenitz and Bucher (1983).

As part of a study to determine the incidence of *A. hydrophila* in foods of animal origin, Palumbo *et al.* (1985) employed some of the media described by von Graevenitz and Bucher (1983) on food samples (various animal products). Preliminary surveys indicated that the clinical media were not suitable for use on food products because they did not allow quantitative recovery of *A. hydrophila* (separate studies indicated that the organism was sensitive to bile salts and sodium desoxycholate), the organism could not be readily differentiated from the microflora of the food, and the microflora of the food overgrew *A. hydrophila* on the plates. This led Palumbo *et al.* (1985) to develop a new medium specifically for isolating *A. hydrophila* from foods. They added ampicillin at a level of 10 mg/L to suppress the background microflora and starch as the differential substance (relatively few organisms in food are amylase positive) to phenol red agar base (Difco). When dilutions of food samples were surface plated on starch ampicillin agar (SAA), colonies of *A. hydrophila* appeared honey-colored, 2–3 mm in diameter after 24 h at 28°C. Amylase production was determined by flooding the plates with *ca* 5 mL of Lugol's iodine; amylase positive colonies were surrounded by a clear zone, indicative of starch hydrolysis. Using this SAA, Palumbo *et al.* (1985) surveyed retail foods of animal origin and found *A. hydrophila* in all food samples examined. They also observed that the number of organisms increased during one week's refrigerated (5°) storage of the food.

Other investigators have utilized SAA to examine various samples for determining the presence of *A. hydrophila*. These include Callister and Agger (1987), who surveyed retail produce, Okrend *et al.* (1987), who sampled retail beef, pork, and poultry, Stern *et al.* (1987), who examined animal fecal specimens and ground beef, and Knochel (1989), who surveyed environmental samples (water, fish, and marine sediment). All investigators found SAA particularly useful, especially in terms of confirmation rates, i.e., presumptive isolates verified as *A. hydrophila*. Knochel (1989) observed an 85% confirmation rate on isolates from food and water.

Confirmation of presumptive *A. hydrophila* isolates is based on a series of biochemical and phenotypic traits (Table 5). These traits will confirm the isolates as *A. hydrophila*, *A. hydrophila* group, or motile aeromonads. Exact speciation (*A. hydrophila*, *A. Sobria*, or *A. caviae*) is based on sugar and other biochemical reactions (Popoff 1984). These can be performed using standard biochemical/bacteriological reactions (Popoff and Veron 1976) or the simplified AH medium of Kaper *et al.* (1979). Several rapid biochemical characterization systems are now available and some have been useful in identifying *A. hydrophila*. These include Minitek (Holmes and Humphry 1988), Mast-ID 15 (Holmes and Dawson

TABLE 5.
BIOCHEMICAL AND PHENOTYPIC TRAITS OF THE *A. HYDROPHILA* GROUP[y]

Gram negative, catalase positive short rod
amylase +
DNase +
oxidase +
resistant to the Vibriostatic agent O/129
beta-hemolytic
motile
gas from glucose[z]
growth at 37°C, optimum at 28°C

[y]Popoff (1984).
[z]*A. caviae* is negative.

1987), and API 20E (Smith *et al.* 1972). A computer based diagnostic model (Robertson and MacLowry 1974) is available for the API 20E system and individual isolates can readily be compared to the reactions of a very large number of cultures, thus aiding in the easy identification of both typical and atypical cultures.

Lachica (see Palumbo *et al.* 1990) has suggested a modification of SAA which eliminates the need for flooding the plates with iodine to identify presumptive colonies of *A. hydrophila*. He used azure amylose (Sigma) in place of soluble starch and observed that amylase positive colonies were surrounded by a light halo against a blue background.

Nishikawa and Kishi (1987) found that *Proteus* obscured the detection of *A. hydrophila* in certain environmental and food samples; addition of starch to a brilliant green-bile salts agar base resulted in inhibition of *Proteus* and detection of *A. hydrophila* by its amylase reaction. However, they gave no indication of the medium's ability to give quantitative recovery of *A. hydrophila*. Palumbo *et al.* (1985) had observed that *A. hydrophila* is sensitive to bile salts. Thus, use of specific media to isolate *A. hydrophila* from foods depends on whether the investigator needs qualitative presence or quantitative recovery and ready confirmation of isolates and what the background microflora of the food is.

RECOVERY OF INJURED CELLS

Observations on the recovery of various pathogenic bacteria from foods have indicated that many of the agents employed in the selective media will inhibit quantitative recovery of bacterial cells damaged (injured) by food processing unit operations such as heating, freezing, drying, sanitizing, and acidifying/fermenting. It is well known that injured cells cannot be quantitatively recovered on selective media (Ray 1989). Smith and Archer (1988) found that many of the selective agents listed in Table 1, e.g, phenylethanol, acriflavin, and potassium tellurite, were detrimental to the recovery of heat-injured *L. monocytogenes*.

Since these selective agents are among the more common inhibitors, food microbiologists have sought alternative media or procedures to insure quantitative recovery of *L. monocytogenes* from processed foods. Various modifications have been suggested. (A) Smith and Buchanan (1990) proposed the addition of a supplement of 0.4% Tween 80, 25 ml/L fetal bovine serum, or 50 mg/L egg yolk tellurite enrichment (Difco) to the MVJ medium. These supplements were able to increase the recovery of heat-injured *L. monocytogenes* approximately 100-fold without the loss of selectivity of the medium. Though there is still some inhibition, these supplements substantially improve the recovery of heat-injured cells. (B) The time of incubation for the enrichment cultures has been increased. Lovett (1988; Lovett *et al.* 1987) described the incubation of the enrichment

broth for 7 days (at 30°C) when used on cheese and other dairy products. The longer enrichment period at the lower temperature (part of the FDA procedure) gave an increased isolation of *L. monocytogenes* from these foods. It was felt that this part of the FDA procedure increased recovery by allowing for repair of injured cells. However, increased incubation time is not a viable option in terms of product quality control procedures. (C) Anaerobic incubation (using the Hungate roll tube technique) increased the recovery of *L. monocytogenes* heated at 62.8°C at least 100-fold over aerobic incubation (Knabel *et al.* 1990).

In contrast to *L. monocytogenes*, there have been very few studies of injury in *A. hydrophila*. Cattabiani and Brindani (1988) studied the effect of chemical sanitizers and observed a high degree of sublethal damage to the cells. This damage was detected as the inability of the injured *A. hydrophila* to grow on selective media (RS medium which contains sodium deoxycholate and novobiocin and MacConkey agar which contains bile salts, neutral red, and crystal violet). Golden *et al.* (1989) studied the effect of heat injury on the ability of *A. hydrophila* to grow under conditions of modified atmosphere storage. They observed that at 30°C, a heat injured culture (exposed to 45°C for 10 min) had a lower growth rate and a smaller total viable population when incubated under CO_2. At 5°C, heat injured cells were markedly affected by CO_2, with the number of viable cells declining during a 12 day holding period. However, Golden *et al.* (1989) did not attempt quantitative recovery of heat injured cells by use of selective media. During their studies of thermal resistance of *A. hydrophila*, Palumbo *et al.* (1987) assumed that there would be heat injury and omitted the ampicillin from SAA, to insure a quantitative recovery of all viable cells present. During their studies on radiation killing of the organism, Palumbo *et al.* (1986) calculated similar D-values for *A. hydrophila* when survivors were plated on either nutrient agar or SAA, suggesting that there were no radiation-injured cells.

CONCLUSION

The isolation procedures and techniques for the foodborne psychrotrophic pathogens *L. monocytogenes* and *A. hydrophila* are similar to those for other foodborne pathogens, i.e., they depend on media which contain selective and differential agents (Tables 1 and 4). The media developed for use with these two organisms give quantitative recovery of 'normal' cells, but special modifications are necessary to recover injured cells. Specific biochemical tests for isolates from the individual media permit confirmation and identification of the cultures isolated. These tests are of extreme importance since foods often contain a complex microflora which can confuse isolation and identification.

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